Potential of Mealybugs Infestation, *Planococcus* spp. (Hemiptera: Pseudococcidae), in an Agroforestry System in Coffee Crops

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Abstract— *The association of tree species to coffee plantations is a common practice in coffee crops, and studies must be performed to establish the effects of these associations. Pests such as the citrus mealybug,* Planococcus citri *(Risso), and the pacificus mealybug,* Planococcus minor *(Maskell) (Hemiptera: Pseudococcidae), can host in several plants and should be studied in relation to this integration. The aim of this study was to evaluate the potential of associated trees to be a source of infestation for coffee crops. The treatments consisted of acrocarpus (*Acrocarpus fraxinifolius*), African mahogany (*Khaya ivorensis*), teak plants (*Tectona grandis*) and macadamia (*Macadamia *sp.), as well as the coffee tree* Coffea Arabica *cv. Mundo Novo. Food preference was studied in laboratory through the test of free choice. Mortality, development and reproduction were also evaluated on each host. Attractiveness of these plants towards the coffee tree was tested by means of an olfactometer, whereby the scale insects were exposed for 15 minutes to the odors of these plants. Both scales settled in all tested plants but the trees did not appear to be suitable hosts. High mortality was found on trees. These scales showed no olfactory preference between the coffee tree and the other tested species and teak leaves had even a repellent effect. It is concluded that acrocarpus, mahogany, macadamia and teak are not potential sources of infestation of mealybugs to the coffee tree, and by consequence they do not represent a threat to the crop.*

Keywords—Planococcus citri, Planococcus minor, Biology, Food Preference, Olfatometry.

I. INTRODUCTION

The use of arboreal species with economic and environmental values can add value to the coffee activity. However, this association requires a detailed knowledge since trees can be a source and refuge of pests and/or the modified environmental conditions, as shading, can affect the incidence of phytophagous arthropods. On the other hand, a diverse agrosystem may have positive impacts where the natural enemies can find refuge, additional food as nectar and pollen, and extra preys, increasing the natural control of pests on coffee (Venzon *et al*., 2014; Tomazella, 2016).

Among these insects, the mealybugs are considered key pests, especially the citrus mealybug, *Planococcus citri* (Risso), and the pacificus mealybug, *Planococcus minor* (Maskell), (Pseudococcidae), which constitute a threat to the coffee plants since they attack flower buds and fruits causing heavy fruit drop (Santa-Cecilia & Souza, 2014). In spite of the diversity of plants that colonize (Williams & Granara de Willink, 1992), these mealybugs may show a certain preference for a host or to have their development and reproduction favored in certain plants due to their nutritional quality.

The insect host selection and recognition process includes several steps, such as habitat and host location, host acceptance and feeding and/or breeding (Le Rü *et al*., 1995b). For this, olfactory, visual, gustatory and tactile stimuli are used, as well as the humidity and intensity of the environment light (Heard, 2000; Powell *et al*., 2006).

Several species of scale insets exploit a limited number of plants, however, they may occasionally occur in other hosts even being not suitable for their development. Mealybugs of the genus *Planococcus*, *Phenacoccus* and other scale insects have sensilla in the antenna with contact and olfactory functions (Salama, 1971; Koteja, 1980; Le Rü *et al*., 1995b; Calatayud & Le Rü, 2006) and it has been hypothesized that they use these structures for the host selection. However, the efficiency to use these sensory organs is limited due to the fact they are apterous (females and males in the first instars) with reduced mobility. Some authors even consider olfactory stimuli of limited value for host location by insects of the order Hemiptera and, such stimuli, would act only at short distances (Backus, 1988). The infestation of new plants would be mainly a passive process, circumscribed to neighboring plants, dispersion by wind, tools used by men or the use of infested plants coming from nurseries. This would result in localized infestations.

In contrast to olfactory stimuli as a mechanism to locate a host, other stimuli may exert some action in the selection (or rejection) of a plant (Le Rü *et al*., 1995b). Olfactory and contact chemoreceptors are present at the apical end of the labium of mealybugs and can be used in the host selection by detecting the stimuli at the leaf surface (Le Rü *et al*., 1995b; Calatayud & Le Rü, 2006). These stimuli may be more important than the olfactory ones present in the antenna. Contact chemoreceptors seem to be used by sucking insects as already verified for aphids. However, it is still under discussion the true role played by volatiles as stimuli for host location in homopteran insects (Powell *et al*., 2006). The host selection by tasting the plant contents through the gustative sensilla present in the cibarium cavity of the alimentary canal is determinant in other sucking insects (Powell *et al*., 2006) and we expect to be similar in mealybugs. Unfortunately to our knowledge there is not studies regarding the presence of gustatory sensilla in the cibarium of mealybugs but we can assume to be similar to other sucking insects.

All these factors may influence the host selection process, however, food preference and plant quality, reflected in the nutritional value, will finally influence the reproduction and the capacity to host the insect.

Thus, the objective of this work was to determine if tree species used in association with coffee plants are potential hosts of two species of mealybugs and by consequence representing a threat to the crop. These studies will ensure a better understanding of the interaction of mealybugs and arboreal species in shaded coffee plantations. The following hypotheses were tested: (a) *P. minor* and *P. citri* mealybugs exhibit dietary preference for coffee plants and have, in this host, better conditions for their development and reproduction; (b) both mealybugs can reproduce in the tested tree species; (c) both mealybugs show olfactory preference for certain plants.

II. MATERIAL AND METHODS

2.1 Mealybugs

Planococcus minor were originally collected in cocoa (*Theobroma cacao*) cv. Comum and *P. citri* in coffee (*Coffea arabica*) cv. Mundo Novo. Both species were reared in laboratory on pumpkins (*Cucurbita maxima* L.) cv. Cabotchá. They were kept in a room, inside wooden cages at 25 ± 2°C and 70 ± 10 RH and in total scotophase.

2.2 Plants

The treatments were constituted by the trees acrocarpus (*Acrocarpus fraxinifolius*), African mahogany (*Khaya ivorensis*), teak (*Tectona grandis*) and macadamia (*Macadamia* sp.). Tree leaves were compared with coffee *C. arabica* cv. Mundo Novo. Tree species were chosen based on the system already implemented in a farm located in Santo Antônio do Amparo, MG, where they are already used for shading coffee plants.

2.3 Food Preference

A free choice test was used to evaluate the preference. Mealybugs were exposed to foliar sections of coffee and a tree in pair comparisons. Leaf sections, with the abaxial side up, were placed on agar (1%) inside Petri dishes of 15 cm diameter. Three foliar sections of each plant were placed alternately and equidistantly, forming a circle. It was used five replicates of each combination and for each species, in a randomized complete block experimental design.

Insects were fasted during one hour before using in the experiment. Fifteen second instars of each species were placed on a circle of filter paper fixed in the center of each plate. These containers were immediately sealed with a plastic film and kept at a room temperature of 25 ± 1 °C and 70 ± 10% RH. The whole set up was covered with black cloth to avoid possible phototropic effect. The evaluations were carried out at 24, 48 and 72 hours counting the number of insects present in each leaf, which was considered as a choice related to food preference. Mealybugs found outside the leaves were not counted.

2.4 Development

A 4-cm diameter leaf section of each tested vegetable was placed inside a 5-cm diameter Petri dish containing a 5 mm layer of agar (1%). Ninety individual first instars of 24 hours-old were collected from the rearing material and placed on the leaf section.

The plates were sealed with plastic film, and dried leaves were replaced when necessary. The plates were placed in room at 25 ± 1 °C and 70 ± 10% RH and total scotophase. The development was followed until emergence of the adults. Mating was assured by isolating a male, already inside the cocoon, and one female in a Petri dish with a plant section inside.

The evaluations were performed daily, recording the duration of the nymphal stage, mortality and the number of viable eggs (according to the hatched nymphs). Ovipositing females were considered as fertile. The experimental design was a completely randomized block design considering one insect as the experimental unit. Initially 90 first instars were used to follow the development but those not found during evaluations were discarded from analysis. Thus, the number of replicates for each treatment is that indicated in the Tables 1 and 2.

2.5 Olfactory response

A four branched olfactory device was used to evaluate the response of both mealybugs to the volatiles emitted by the trees face to those of coffee (Vet *et al.*, 1983). The source of odors originated from freshly leaves kept inside a 400 cc glass container. Air flux was calibrated to 1200 mL/min so each branch received 300 mL/min of air. Coffee and tree odors occupied one branch each while purified air occupied the two other branches. They were positioned at random in each branch of the olfactometer. Individual mealybug of third instar was exposed for 15 minutes to the odors of the hosts testing 30 insects for each combination. A choice for an odor was defined when the insect surpassed a mark located at 2 cm from the releasing point toward a branch.

The residence time in each branch was recorded by means of the software JWatcher vs 1.0. After 10 tests, the leaves were replaced and the olfatometer, washed with detergent, water and ethanol 70%. The tests were conducted in an environment without any visual interference.

2.6 Data analysis

Data from the choice test was analyzed by means of the Chi-Square (χ^2) test considering the observed and expected frequencies. Data from nymphal mortality was analyzed by the Chi square test (χ^2). The duration of nymphal stage was only analyzed for *P. citri* by using the Student Test with data transformed to $\sqrt{x}$, because the high mortality impeded to make more than one comparison. For the same reason no statistical analysis was possible to compare the number of viable eggs.

The Chi-square test (α=0.05) was used for pair comparisons of the final choice. Means of the total time in each branch was submitted to Analysis of Variance and were compared by the Tukey test ($p \leq 0.05$), with data transformed in arcsin$\sqrt{x/100}$. The number of nymphs that did not respond (undecided) and remained in the neutral zone of the olfactometer were recorded but not considered for analysis.

III. RESULTS AND DISCUSSION

3.1 Food Preference

Some food preference was found in the choice test between the offered hosts (Figs. 1-4). *Planococcus citri* showed preference for coffee face to teak and macadamia. Mealybugs abandoned these hosts after 24 and 48 hours, a sufficient time to taste the phloem sap suggesting a repellent effect. Coffee, mahogany and acrocarpus were equally preferred. *Planococcus minor* also avoided teak and macadamia and settled on coffee. Mahogany was equally preferred face to coffee and acrocarpus showed to be very attractive to this mealybug.

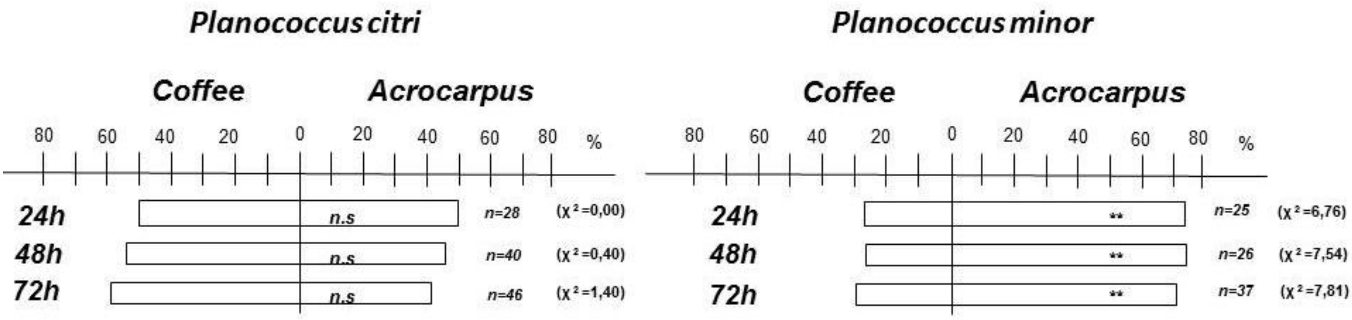

FIG. 1. Free-choice test. Differences according to the χ^2 test with 1 d.f. at 5% (χ^2 value= 3.84) (n = number of insects with choice).

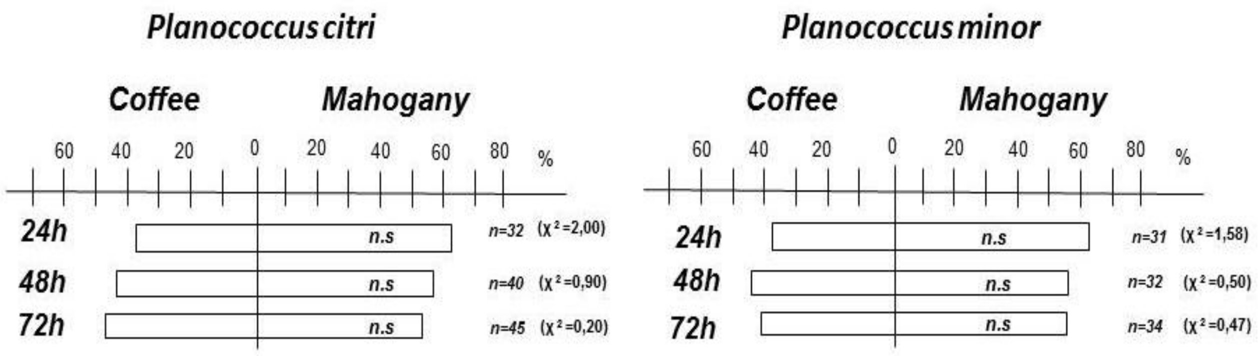

FIG. 2. Free-choice test. Differences according to the χ^2 test with 1 d.f. at 5% (χ^2 value= 3.84) (n = number of insects with choice).

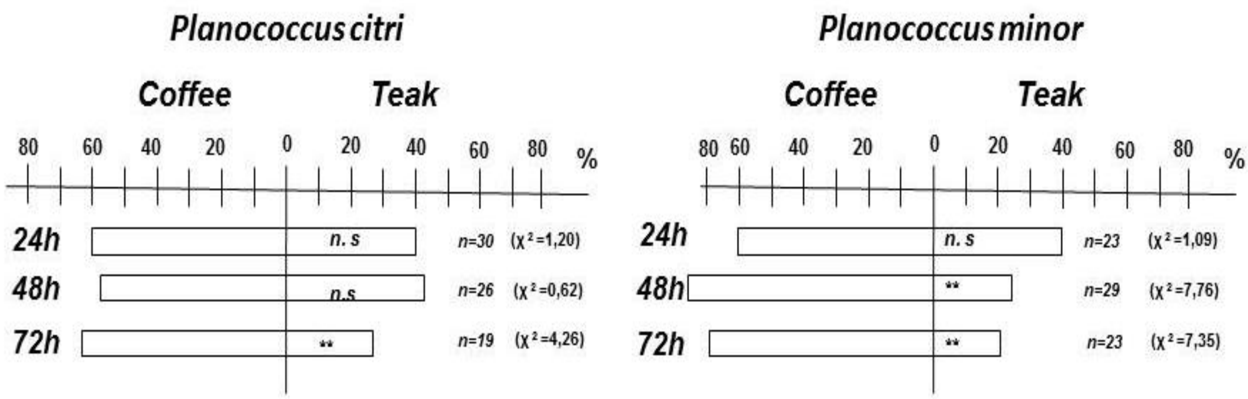

FIG. 3. Free-choice test. Differences according to the χ^2 test with 1 d.f. at 5% (χ^2 value= 3.84) (n = number of insects with choice).

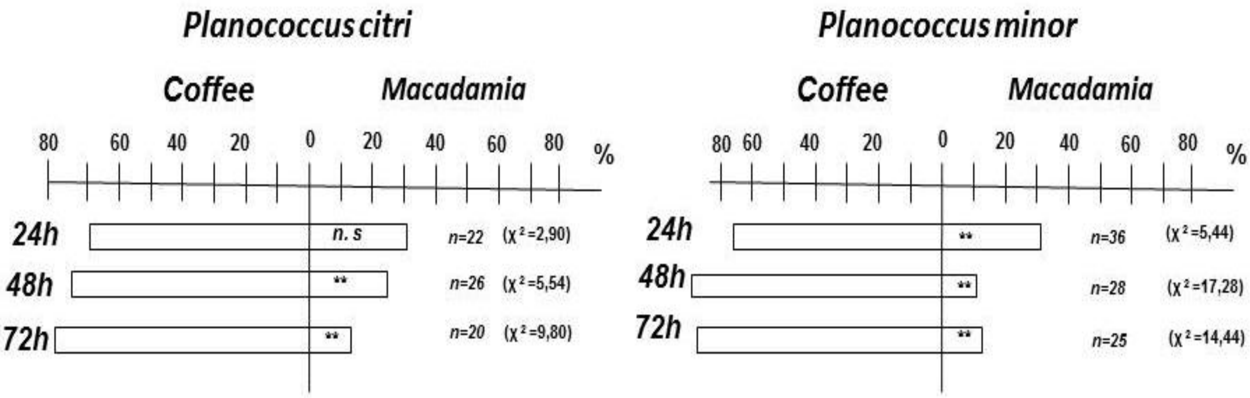

FIG. 4. Free-choice test. Differences according to the χ^2 test with 1 d.f. at 5% (χ^2 value= 3.84) (n = number of insects with choice).

3.2 Development

A high mortality was found in *P. citri* and *P. minor* in all tested trees, excepting in coffee, with values above 75% (Tables 1 and 2). The low number of emerged females impeded to evaluate other biological parameters related to the effect of the substrate (host).

TABLE 1
DEVELOPMENT OF *Planococcus citri* IN DIFFERENT HOSTS (25±1°C, 70±10% RH, total scotophase).

Host	Nymph mortality (%)	Nymph period of females (days)	Number of viable eggs/fertile female
Coffee *Coffea arabica*	50.0 b (n=46)	24.0±1.1 b (n=23)	39.4±10.6 (1) (n=8)
Acrocarpus *Acrocarpus fraxinifolius*	75.8 b (n=33)	32.3±0.8 a (n=8)	18.0±0.0 (1) (n=1)
Mahogany *Khaya ivorensis*	98.3 a (n=58)	22.0±0.0 (1) (n=1)	(1)
Teak *Tectona grandis*	100.0 a (n=41)	0.0 (1)	(1)
Macadamia *Macadamia* sp.	96.0 a (n=50)	19.0±0.0 (1) (n=2)	(1)
p value	≤ 0.001 (χ^2)	≤ 0.001 (Student) (2)	

(1) Not considered for statistical analysis; (2) Means followed by the same letter in the column are not different according to the Chi square (χ^2) and Student Test. Data transformed to √x; n = number of insects.

TABLE 2
DEVELOPMENT OF *Planococcus minor* IN DIFFERENT HOSTS (25±1°C, 70±10% RH, total scotophase).

Host	Nymph mortality (%)	Nymph period of females (days)	Number of viable eggs/fertile female
Coffee *Coffea arabica*	48.8 b (n=41)	24.0±1.1 (1) (n=21)	28.4 ± 13.6 (1) (n=5)
Acrocarpus *Acrocarpus fraxinifolius*	97.2 a (n=36)	35.0±0.0 (1) (n=1)	(1)
Mahogany *Khaya ivorensis*	92.7 a (n=67)	16.4±1.4 (1) (n=5)	(1)
Teak *Tectona grandis*	100.0 a (n=53)	0.0 (1)	(1)
Macadamia *Macadamia* sp.	100.0 a (n=43)	0.0 (1)	(1)
p value	≤ 0.001 (χ^2)		

(1) Not considered for statistical analysis; (2) Means followed by the same letter in the column are not different according to the Chi square (χ^2). Data transformed to √x; n = number of insects.

3.3 Olfactory response

3.3.1 Response to coffee plants and acrocarpus

Coffee, acrocarpus and clean air hosted similar number of mealybugs. So, the supposed volatile compounds emitted by acrocarpus or coffee trees were neither attractive nor repellent for both mealybug species (Table 3). The total residence time of *P. citri* in each branch was similar. *Planococcus minor* stayed for similar time in both host branches and little longer in blank air.

TABLE 3
FINAL CHOICE 3rd INSTARS OF *Planococcus citri* AND *Planococcus minor* IN OLFACTOMETER (4 BRANCHES) (N=30) (15 MINUTES).

Insect	Olfactory response			Combination		
	Coffee (branch 1)	Acrocarpus (branch 2)	Clean air (branches 3 & 4)	Without response	1 *vs* 2	(1+2) *vs* (3+4)
P. citri	6	8	10	6 (20%)	0.6 n.s	0.7 n.s
P. minor	5	8	16	1 (3.3%)	0.8 n.s	0.3 n.s

Differences according to the Chi square test (χ^2) (α=0.05), (N= number of insects).

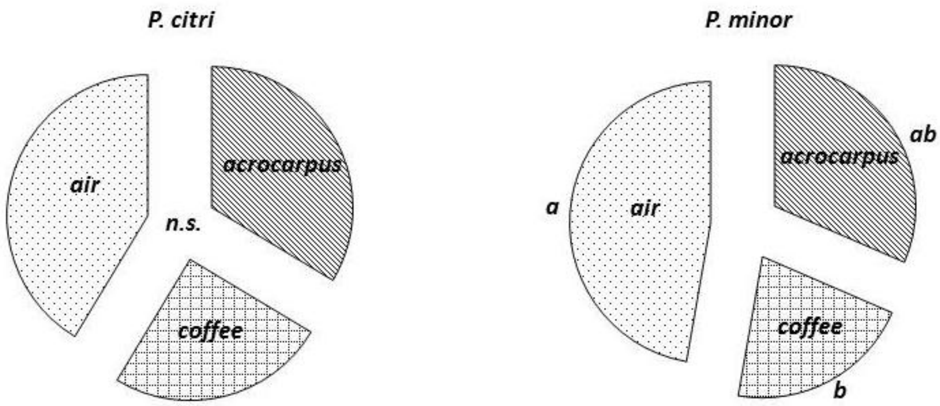

FIG. 5. Mean relative time (%) spent in each field in an olfactometer by 3rd instar nymphs of *P. citri* and *P. minor* exposed to three odors. ANOVA values: p=0.207, n=27 and p=0.026, n=30, respectively. Data transformed to arcsin √x/100. Means followed by the same letter are not different according to Anova followed by the Test of Tukey; n.s = no significant.

3.3.2 Response to coffee plants and mahogany

Planococcus citri nymphs showed no preference for the offered odors, while those of *P. minor* showed olfactory preference for mahogany face to coffee. Air was not more attractive than plant odors (Table 4). Nymphs of *P. citri* remained longer in clean air. Nymphs of *P. minor* remained longer in the air and mahogany, and shorter period in coffee (Fig. 6).

TABLE 4
FINAL CHOICE 3rd INSTARS OF *Planococcus citri* AND *Planococcus minor* IN OLFACTOMETER (4 BRANCHES) (N=30) (15 MINUTES).

Insect	Olfactory response			Combination		
	Coffee (branch 1)	Mahogany (branch 2)	Clean air (branches 3 & 4)	Without response	1 *vs* 2	(1+2) *vs* (3+4)
P. citri	6	6	16	2 (6.7%)	0.3 n.s	0.6 n.s
P. minor	0	8	15	7 (23.3%)	**6.6***	2.1 n.s

Differences according to the Chi square test (χ^2) (α=0.05), (N= number of insects).

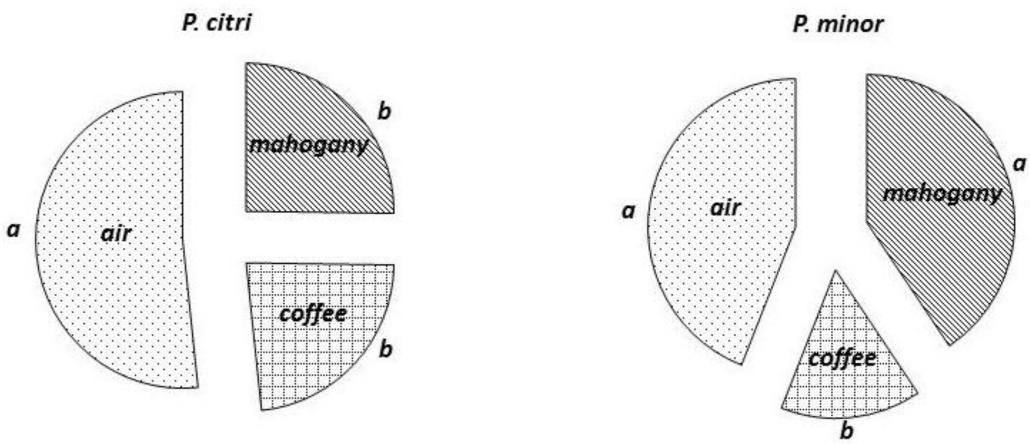

FIG. 6. Mean relative time (%) spent in each field in an olfactometer by 3rd instar nymphs of *P. citri* and *P. minor* exposed to three odors. ANOVA values: p=0.005, n=28 and p=0.002, n=30, respectively. Data transformed to arcsin √x/100. Means followed by the same letter are not different according to Anova followed by the Test of Tukey.

3.3.3 Response to coffee plants and teak

Teak appeared to have a repellent effect only for *P. citri* since insects were attracted to coffee and air (Table 5), but the permanency time was similar in all braches (Fig. 7).

TABLE 5
FINAL CHOICE 3rd INSTARS OF *Planococcus citri* AND *Planococcus minor* IN OLFACTOMETER (4 BRANCHES) (N=30) (15 MINUTES).

Insect	Olfactory response			Combination		
	Coffee (branch 1)	Teak (branch 2)	Clean air (branches 3 & 4)	Without response	1 *vs* 2	(1+2) *vs* (3+4)
P. citri	11	4	8	7 (23.3%)	**5.3 ***	2.1 n.s
P. minor	7	7	14	2 (6.7%)	0.0 n.s	0.0 n.s

Differences according to the Chi square test (χ^2) (α=0.05), (N= number of insects).

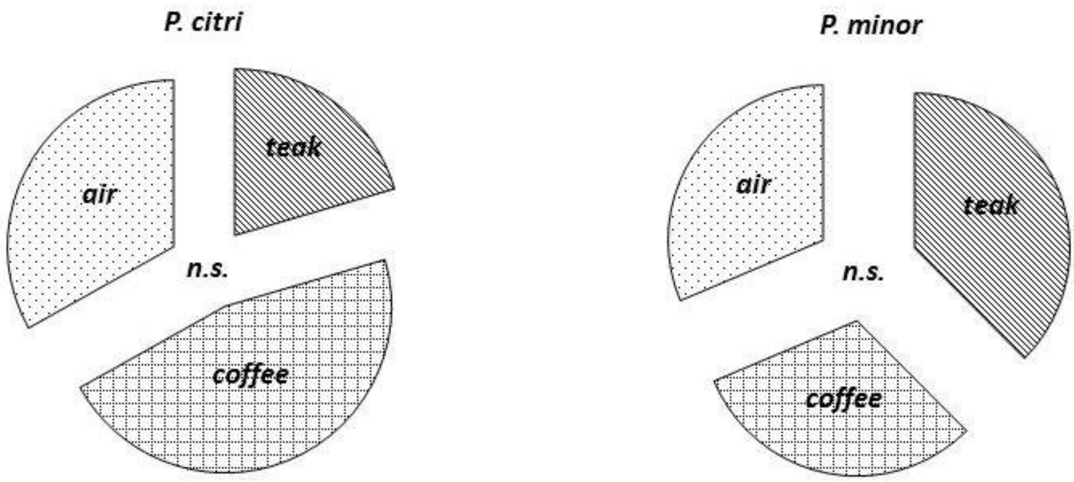

FIG. 7. Mean relative time (%) spent in each field in an olfactometer by 3rd instar nymphs of *P. citri* and *P. minor* exposed to three odors. ANOVA values: p=0.063, n=22 and p=0.775, n=30, respectively. Data transformed to arcsin √x/100. Means followed by the same letter are not different according to Anova followed by the Test of Tukey; n.s = no significant.

3.3.4 Response to coffee plants and macadamia

Coffee and macadamia odors had no effect on any of the mealybugs, which were equally distributed in olfactometer branches (Table 6). The permanency time inside each branch neither showed differences between odors (Fig. 8).

TABLE 6
FINAL CHOICE 3rd INSTARS OF *Planococcus citri* AND *Planococcus minor* IN OLFACTOMETER (4 BRANCHES) (N=30) (15 MINUTES).

Insect	Olfactory response			Combination		
	Coffee (branch 1)	Macadamia (branch 2)	Clean air (branches 3 & 4)	Without response	1 *vs* 2	(1+2) *vs* (3+4)
P. citri	6	6	9	9 (30%)	0.2 n.s	0.4 n.s
P. minor	6	7	11	6 (20%)	0.2 n.s	0.2 n.s

Differences according to the Chi square test (χ^2) (α= 0.05), (N= number of insects).

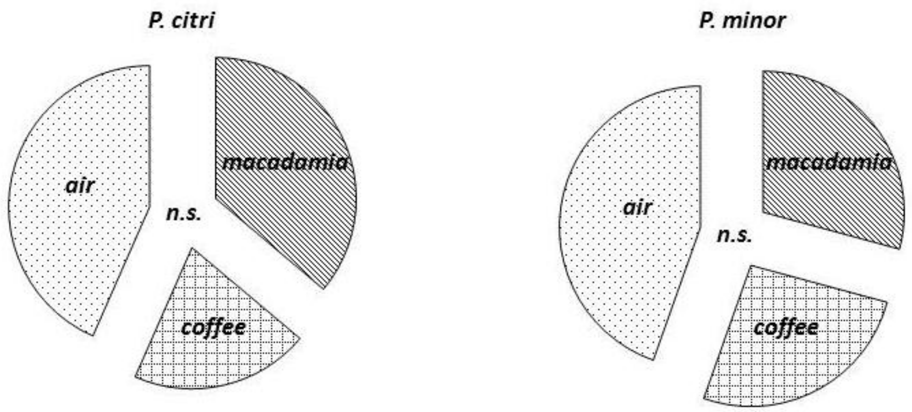

FIG. 8. Mean relative time (%) spent in each field in an olfactometer by 3rd instar nymphs of *P. citri* and *P. minor* exposed to three odors. ANOVA values: p=0.169, n=22 and p=0.137, n=25, respectively. Data transformed to arcsin √x/100. Means followed by the same letter are not different according to Anova followed by the Test of Tukey; n.s = no significant.

If we accept that tasting plant contents (cell or phloem sap) by ingesting plant fluids should be the main mechanism for plant selection, the free choice test should give a good insight about plant suitability. Mealybugs take a long time before reaching the phloem and ingest phloem sap (Santa-Cecilia *et al*., 2013), so plant exposing to these insects should last long period, 72 hours in this test, to get reliable results.

This study showed that, despite of the mealybugs were able to settle in the tested trees, there are different responses when compared with coffee plants. Settling or feeding in a plant does not mean that the plant is adequate and can support an insect colony. Plant nutrients can be suboptimal for reproduction (Le Rü *et al*., 1995a) and restrains colony size. Our data indicated that neither acrocarpus nor mahogany have a repellent effect for mealybugs but they seem to be poor hosts due to the high mortality. Data from the olfactometer are in agreement with these results.

Teak did not appear to be a good host in all tests. Data from the choice test showed a repellent effect and rearing on leaves showed a high mortality.

Plant selection process is a sequence of steps involving different environmental and plant stimulus. The olfactory response is one of these steps. All responses are related to the degree of adaptation of the insect to the host (Moura *et al*., 1991). Despite the reports about the presence of olfactory receptors in the mealybug antenna (Salama, 1971; Koteja, 1980; Le Rü *et al*., 1995a; Calatayud & Le Rü, 2006), we ignore the role they play in plant selection since the nymphs and adult females are apterous with little option to search and select a host.

Both tested mealybug species are able to colonize diverse plants since they are polyphagous. Macadamia has been reported as host for *P. citri*, and macadamia and teak for *P. minor* (García Morales *et al*., 2016). However, in the study presented here they did not appear as acceptable hosts for these mealybugs.

Cacao plants are usually colonized by *P. minor* and in less extension for *P. citri* suggesting the former should be more selective. Our results did not showed difference between species although cacao was not tested in this study.

This study showed that the tested trees, usually associated to coffee crops, are not suitable hosts for both species of mealybugs and they would not be source of infestation for coffee crops. It should be noted that this study was performed in laboratory, under controlled conditions, and field conditions could change the mealybug behavior according to environmental conditions.

IV. CONCLUSION

Acrocarpus, mahogany, teak and macadamia are not suitable hosts for *P. citri* and *P. minor* and they should not be source of infestation when associated to coffee crops.

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